Litton BIONETICS

MUTAGENIC EVALUATION
OF COMPOUND

FDA 75-49
000109-94-4
ETHYL FORMATE
(FOOD GRADE)

5516 Nicholson Lane
Kensington, Maryland
20795

Mutagenic Evaluation of Compound FDA 75-49 **Ethyl Formate (Food Grade)**
5/31/76

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MUTAGENIC EVALUATION OF COMPOUND

FDA 75-49
000109-94-4
ETHYL FORMATE
(FOOD GRADE)

SUBMITTED TO

FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
ROCKVILLE, MARYLAND 20852

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

MAY 31, 1976

Litton BIONETICS

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EVALUATION SUMMARY

Compound FDA 75-49, Ethyl Formate (Food Grade), did not exhibit genetic activity in any of the *in vitro* assays employed in this evaluation.

DATE: May 31, 1976

SPONSOR: U.S. Food and Drug Administration, Contract Number 223-74-2104

SUBJECT: Evaluation of Test Compound Ethyl Formate (Food Grade), FDA 75-49

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: March 10, 1976
2. Description: Colorless liquid

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae* strain D4

Bacteria Strains: *Salmonella typhimurium* strains TA-1535
TA-1537
TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	6 µmoles
2. Isocitric acid	35 µmoles
3. Tris buffer, pH 7.4	28 µmoles
4. $MgCl_2$	2 µmoles
5. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for survival against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^9 cells from a log phase culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests the 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic tissue culture plates. Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C in an oxygen atmosphere with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

E. Data Recording and Reporting

1. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program.

2. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were transferred directly to the report form sheets and presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: FDA 75-49 Ethyl Formate (Food Grade)

2. Test solvent: DMSO

3. Solubility of the test compound under treatment conditions: Soluble

4. Additional comments: Colorless liquid

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: March 29, 1976

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	1.25	1.25
1/2 50% Survival	2.50	2.50
50% Survival	5.00	5.00

C. Suspension Assay Results

The suspension test results for the test compound are summarized in the following four tables. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the set presents the results for the nonactivation assays, and the second through the fourth table of the set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 05/26/76

SPECIES / NONACTIVATION COMPOUND 000109944

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		17.45	11.79	15.10	43.56	21.89	CONTROLS
NAP		221.77	339.29	109.48	154.52	143.62	
NA1		8.82	16.55	17.35	47.69	25.67	TEST DATA
NA2		9.28	13.01	15.77	30.49	20.62	
NA3		10.54	9.93	15.80	34.93	22.63	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 05/26/76

SPECIES ICRFLO/MOUSE COMPOUND 000109944

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	24.89	2.53	11.68		29.78	21.30	
ACT	A-C	26.14	4.55	14.65		24.96	23.04	NEGATIVE CONTROLS
ACT	ALI	19.23	4.49	18.36	16.13	28.18	30.41	
ACT	ALU	17.71	7.62	16.64		24.23	32.15	
ACT	PLI	290.31	170.63	160.10		57.98	50.11	
ACT	PLU	20.74	6.46	38.18		35.90	41.03	POSITIVE CONTROLS
ACT	LI1	13.93	10.74	65.52	22.34	26.21	33.57	
ACT	LI2	27.37	9.52	21.93		20.77	25.07	
ACT	LI3	23.53	5.08	17.03		26.21	37.02	
ACT	LU1	20.31	7.11	21.82		32.66	33.39	TEST DATA
ACT	LU2	20.23	7.05	25.12		23.81	24.79	
ACT	LU3	21.33	6.57	15.72		20.77	33.08	

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LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 05/26/76

SPECIES SPRDAW/RAT COMPOUND 000109944

EST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA1538 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	14.53	4.42		13.79			16.11	19.89	
ACT	A-C	14.67	4.38		13.34			24.38	18.36	NEGATIVE CONTROLS
ACT	ALI	22.31	14.48	8.41	2.78	20.90	4.26	22.57	18.67	
ACT	ALU	16.26	49.91	6.28	25.90	11.62		16.49	16.81	
ACT	PLI	82.70	266.27		186.05			50.00	34.52	POSITIVE CONTROLS
ACT	PLU	17.62	60.04		102.64			20.75	18.70	
ACT	LI1	20.13	77.20	4.24	33.09		7.20	16.08	16.47	
ACT	LI2	22.70	41.92	5.45	15.70			18.33	18.71	
ACT	LI3	27.63	22.48		26.30		4.58	24.55	17.82	TEST DATA
ACT	LU1	16.65	21.69	5.30	17.56			11.44	9.31	
ACT	LU2	19.11	60.34	3.50	54.72	9.86		20.75	19.46	
ACT	LU3	20.64	35.81		53.76	10.45		16.48	17.69	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 05/26/76

SPECIES RHESUS/MONKEY COMPOUND 000109944

TEST	ORG	TA1535 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	15.46		5.43	13.89	17.69	16.85	
ACT	A-C	15.20		4.38	13.25	17.44	16.07	NEGATIVE CONTROLS
ACT	ALI	14.41	4.92	3.74	14.44	22.41	18.05	
ACT	ALU	16.08		4.81	10.05	19.62	16.62	
ACT	PLI	81.60	81.60	252.81	299.86	67.05	20.19	POSITIVE CONTROLS
ACT	PLU	15.37		8.00	13.35	16.92	17.76	
ACT	LI1	13.19		7.45	15.61	17.24	14.94	
ACT	LI2	14.20		4.98	15.29	16.85	12.24	
ACT	LI3	33.76	17.46	3.56	14.21	23.80	17.38	TEST DATA
ACT	LU1	15.23		6.28	14.67	19.01	17.91	
ACT	LU2	14.22		5.52	16.39	17.96	14.52	
ACT	LU3	15.36		4.51	14.06	19.49	15.31	

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DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI = Activation: Homogenate Control (Liver) ALU = Activation: Homogenate Control (Lung) (ALI, ALU) or A+T ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (Macaca mulatta)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. SUMMARY OF TEST RESULTS

Plate Tests

A. Name or code designation of the test compound: FDA 75-49, Ethyl Formate (Food Grade)

B. Test date: May 17, 1976

C. Concentrations of the test compound: (1) 5% (2) 2.5% (3) 1.25%

Test	Species	Tissue	REVERTANTS/PLATE TA-1535 1	TA-1535 2	TA-1537 1	TA-1537 2	TA-1538 1	TA-1538 2
1. Non-activation								
Solvent Control[a]	---	---	13	13	9	13	14	12
Positive Control[b]	---	---	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
Test Compound	---	---						
(1)	---	---	19	15	15	14	16	10
(2)	---	---	10	11	13	13	11	12
(3)	---	---	9	10	9	9	12	10
2. Activation								
Solvent Control[a]	Mouse	Liver	23	26	13	12	15	17
	Rat	Liver	16	10	12	8	16	14
	Monkey	Liver	28	34	12	14	14	18
Positive Control[b]	Mouse	Liver	220	237	200	250	375	380
	Rat	Liver	226	254	260	200	$>10^3$	$>10^3$
	Monkey	Liver	140	138	234	360	$>10^3$	$>10^3$
Test Compound								
(1)	Mouse	Liver	15	8	13	18	21	15
(2)	Mouse	Liver	16	14	15	11	14	13
(3)	Mouse	Liver	14	19	12	13	11	12
(1)	Rat	Liver	14	10	8	11	12	13
(2)	Rat	Liver	12	12	10	7	11	10
(3)	Rat	Liver	17	12	10	10	9	12
(1)	Monkey	Liver	17	9	15	17	13	17
(2)	Monkey	Liver	12	18	13	14	12	17
(3)	Monkey	Liver	23	21	12	13	15	14

[a] Nonactivation assays consist of the cells plus the test compound vehicle (solvent). For activation assays, the overlay contains the activation system plus the test compound vehicle.

[b]

Nonactivation			Activation		
TA-1535	EMS	10 µl/plate	TA-1535	ANTH	100 µg/plate
TA-1537	QM	20 µg/plate	TA-1537	AMQ	100 µg/plate
TA-1538	NF	100 µg/plate	TA-1538	AAF	100 µg/plate

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound FDA 75-49, Ethyl Formate (Food Grade), was tested for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. *Salmonella typhimurium*

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative. The LI1 dose with TA-1538 using mouse tissue was repeated because of a low population count. Doses 1 and 2 using rat liver and lung tissue in the test with TA-1537, and the LI1, LI3, LU2, and LU3 doses with TA-1538 using rat tissue, were repeated because of increased mutation frequencies. The LI3 dose with TA-1535 using monkey tissue was repeated because of an increased mutant frequency. All repeat tests were negative. The rat ALI control for TA-1538 was approximately a log lower than expected based on the other control frequencies. Inspection of the raw data showed that the population counts for TA-1538 ALI were a log higher than other comparable data, and the number of revertants was consistent with a dilution/plating error. Therefore, the test data were compared to the A-C, A+C, and ALU controls in this case.

B. *Saccharomyces cerevisiae*

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

Compound FDA 75-49, Ethyl Formate (Food Grade), did not exhibit genetic activity in any of the assays employed in this investigation.

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Department of Genetics

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Vice President

VII. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Interpretation of Results

Dose-related increases in mutant counts is the most reliable method to demonstrate mutagenicity. Mutant increases at only one or two doses may be significant if they occur at the higher doses. Increases at low or intermediate concentrations followed by reduced mutant counts at higher doses may indicate that the test chemical has a narrow activity range or that the high dose levels were toxic and the induced revertant cells were killed. We are able to detect the latter possibility by inspecting the background growth, and the former possibility can be investigated by looking at a narrow series of dose levels bracketing the presumptive active range.

It is difficult to detect mutagens in this assay with little or no toxicity since such agents are generally weak mutagens and produce only 2-3 fold increases in mutant counts. Variations of 2-3 fold are often within normal fluctuations of the spontaneous counts, and the use of even higher concentrations is often difficult because of the likelihood of overloading the system with large quantities of the chemical. To resolve the mutagenicity of such a chemical, other assays to which statistical evaluations can be applied may be necessary.

VIII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the *his*$^-$ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

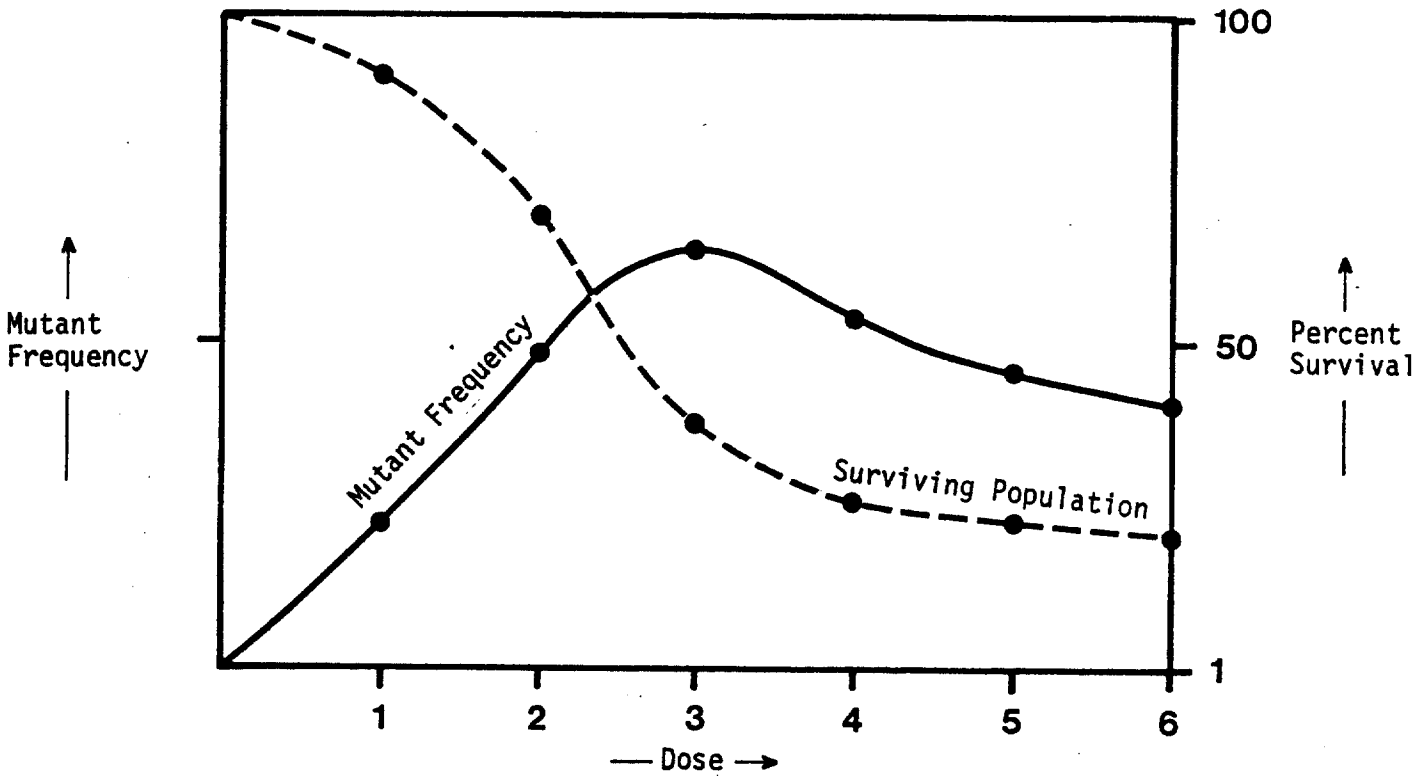
D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT	OBSERVED DOSE RESPONSE
(1) Dose levels 1,2 & 3 were used	A typical positive dose response set of data would be obtained.
(2) Dose levels 2, 3 & 4 were used	The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
(3) Dose levels 3, 4 & 5 were used	Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX

Tabulation of Data

Litton BIONETICS

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 612717 DETECTOR TA1535 SPECIES / DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	2435	0425	17.45	0
	NAP		EMS 0.2%	2301	5103	221.77	0
000109944	NA1		0005-0 PCT.	2439	0215	8.82	0
000109944	NA2		0025-1 PCT.	2457	0228	9.28	0
000109944	NA3		0125-2 PCT.	2534	0267	10.54	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 612718 DETECTOR TA1537 SPECIES / DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	1026	0121	11.79	0
	NAP		QM 13 UG/ML	0560	1900	339.29	0
000109944	NA1		0005-0 PCT.	0701	0116	16.55	0
000109944	NA2		0025-1 PCT.	0684	0089	13.01	0
000109944	NA3		0125-2 PCT.	0685	0068	9.93	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 612101 DETECTOR TA1538 SPECIES / DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	1066	0161	15.10	0
	NAP		NF 667 UG/ML	1350	1478	109.48	0
000109944	NA1		0005-0 PCT.	0876	0152	17.35	0
000109944	NA2		0025-1 PCT.	0856	0135	15.77	0
000109944	NA3		0125-2 PCT.	0785	0124	15.80	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 613403 DETECTOR 0000D4 SPECIES / DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	0932	0406	0204	43.56	21.89	0
	NAP		EMS 1.0 %	0862	1332	1238	154.52	143.62	0
000109944	NA1		0005-0 PCT.	0822	0392	0211	47.69	25.67	0
000109944	NA2		0025-1 PCT.	1125	0343	0232	30.49	20.62	0
000109944	NA3		0125-2 PCT.	1171	0409	0265	34.93	22.63	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 613302 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	2073	0516	24.89	0
	A-C		SOLVENT	1859	0486	26.14	0
	ALI		TISSUE	1971	0379	19.23	0
	ALU		TISSUE	2270	0402	17.71	0
	ACP	LI	DMN 90 UM/ML	1857	5391	290.31	0
	ACP	LU	DMN 90 UM/ML	1914	0397	20.74	0
000109944	ACT	LI1	0005-0 PCT.	0955	0133	13.93	0
000109944	ACT	LI2	0025-1 PCT.	2159	0591	27.37	0
000109944	ACT	LI3	0125-2 PCT.	2299	0541	23.53	0
000109944	ACT	LU1	0005-0 PCT.	2359	0479	20.31	0
000109944	ACT	LU2	0025-1 PCT.	2650	0536	20.23	0
000109944	ACT	LU3	0125-2 PCT.	2396	0511	21.33	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 613101 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1342	0034	2.53	0
	A-C		SOLVENT	1230	0056	4.55	0
	ALI		TISSUE	0512	0023	4.49	0
	ALU		TISSUE	0998	0076	7.62	0
	ACP	LI	AMQ 333 UG/ML	0446	0761	170.63	0
	ACP	LU	AMQ 333 UG/ML	1115	0072	6.46	1
000109944	ACT	LI1	0005-0 PCT.	0596	0064	10.74	0
000109944	ACT	LI2	0025-1 PCT.	0830	0079	9.52	0
000109944	ACT	LI3	0125-2 PCT.	0708	0036	5.08	0
000109944	ACT	LU1	0005-0 PCT.	0816	0058	7.11	1
000109944	ACT	LU2	0025-1 PCT.	0979	0069	7.05	0
000109944	ACT	LU3	0125-2 PCT.	1051	0069	6.57	2

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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 613301 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1455	0170	11.68	0
	A-C		SOLVENT	1242	0182	14.65	0
	ALI		TISSUE	0708	0130	18.36	0
	ALU		TISSUE	1160	0193	16.64	0
	ACP	LI	ANTH 67 UG/ML	0797	1276	160.10	0
	ACP	LU	ANTH 67 UG/ML	1024	0391	38.18	0
000109944	ACT	LI1	0005-0 PCT.	0145	0095	65.52	0
000109944	ACT	LI2	0025-1 PCT.	0684	0150	21.93	0
000109944	ACT	LI3	0125-2 PCT.	0828	0141	17.03	0
000109944	ACT	LU1	0005-0 PCT.	0637	0139	21.82	0
000109944	ACT	LU2	0025-1 PCT.	0828	0208	25.12	0
000109944	ACT	LU3	0125-2 PCT.	1164	0183	15.72	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 614207 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	0688	0111	16.13	0
000109944	ACT	LI1	0005-0 PCT.	0488	0109	22.34	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 614201 DETECTOR 0000D4 SPECIES ICRFLO/MOUSE DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0601	0179	0128	29.78	21.30	0
	A-C		SALINE	0625	0156	0144	24.96	23.04	0
	ALI		TISSUE	0628	0177	0191	28.18	30.41	0
	ALU		TISSUE	0619	0150	0199	24.23	32.15	0
	ACP	LI	DMN 90 UM/ML	0902	0523	0452	57.98	50.11	0
	ACP	LU	DMN 90 UM/ML	0819	0294	0336	35.90	41.03	0
000109944	ACT	LI1	0005-0 PCT.	0557	0146	0187	26.21	33.57	0
000109944	ACT	LI2	0025-1 PCT.	0698	0145	0175	20.77	25.07	0
000109944	ACT	LI3	0125-2 PCT.	0786	0206	0291	26.21	37.02	0
000109944	ACT	LU1	0005-0 PCT.	0542	0177	0181	32.66	33.39	0
000109944	ACT	LU2	0025-1 PCT.	0714	0170	0177	23.81	24.79	0
000109944	ACT	LU3	0125-2 PCT.	0780	0162	0258	20.77	33.08	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 613803 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	2526	0367	14.53	0
	A-C		SOLVENT	2270	0333	14.67	0
	ALI		TISSUE	0789	0176	22.31	0
	ALU		TISSUE	2036	0331	16.26	0
	ACP	LI	DMN 90 UM/ML	2780	2299	82.70	0
	ACP	LU	DMN 90 UM/ML	2242	0395	17.62	0
000109944	ACT	LI1	0005-0 PCT.	0447	0090	20.13	0
000109944	ACT	LI2	0025-1 PCT.	0304	0069	22.70	0
000109944	ACT	LI3	0125-2 PCT.	0952	0263	27.63	0
000109944	ACT	LU1	0005-0 PCT.	1646	0274	16.65	0
000109944	ACT	LU2	0025-1 PCT.	1565	0299	19.11	0
000109944	ACT	LU3	0125-2 PCT.	1754	0362	20.64	0

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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 613401 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	2034	0090	4.42	0
	A-C		SOLVENT	2007	0088	4.38	0
	ALI		TISSUE	0829	0120	14.48	0
	ALU		TISSUE	0533	0266	49.91	0
	ACP	LI	AMQ 333 UG/ML	0255	0679	266.27	0
	ACP	LU	AMQ 333 UG/ML	0548	0329	60.04	0
000109944	ACT	LI1	0005-0 PCT.	0329	0254	77.20	0
000109944	ACT	LI2	0025-1 PCT.	0427	0179	41.92	0
000109944	ACT	LI3	0125-2 PCT.	0703	0158	22.48	0
000109944	ACT	LU1	0005-0 PCT.	1079	0234	21.69	0
000109944	ACT	LU2	0025-1 PCT.	0353	0213	60.34	0
000109944	ACT	LU3	0125-2 PCT.	0444	0159	35.81	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 614206 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	0416	0035	8.41	0
	ALU		TISSUE	0382	0024	6.28	0
000109944	ACT	LI1	0005-0 PCT.	0236	0010	4.24	0
000109944	ACT	LI2	0025-1 PCT.	0440	0024	5.45	0
000109944	ACT	LU1	0005-0 PCT.	0283	0015	5.30	0
000109944	ACT	LU2	0025-1 PCT.	0429	0015	3.50	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 613405 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1400	0193	13.79	0
	A-C		SOLVENT	0667	0089	13.34	0
	ALI		TISSUE	5189	0144	2.78	0
	ALU		TISSUE	0776	0201	25.90	0
	ACP	LI	ANTH 67 UG/ML	1061	1974	186.05	0
	ACP	LU	ANTH 67 UG/ML	0605	0621	102.64	0
000109944	ACT	LI1	0005-0 PCT.	0553	0183	33.09	0
000109944	ACT	LI2	0025-1 PCT.	0866	0136	15.70	0
000109944	ACT	LI3	0125-2 PCT.	0616	0162	26.30	0
000109944	ACT	LU1	0005-0 PCT.	0541	0095	17.56	0
000109944	ACT	LU2	0025-1 PCT.	0530	0290	54.72	0
000109944	ACT	LU3	0125-2 PCT.	0599	0322	53.76	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 614204 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	0737	0154	20.90	0
	ALU		TISSUE	1196	0139	11.62	0
000109944	ACT	LU2	0025-1 PCT.	1187	0117	9.86	0
000109944	ACT	LU3	0125-2 PCT.	1301	0136	10.45	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 614601 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	1548	0066	4.26	0
000109944	ACT	LI1	0005-0 PCT.	1000	0072	7.20	0
000109944	ACT	LI3	0125-2 PCT.	2120	0097	4.58	0

Litton BIONETICS

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 614104 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0875	0141	0174	16.11	19.89	0
	A-C		SALINE	1013	0247	0186	24.38	18.36	0
	ALI		TISSUE	0873	0197	0163	22.57	18.67	0
	ALU		TISSUE	0940	0155	0158	16.49	16.81	0
	ACP	LI	DMN 90 UM/ML	0872	0436	0301	50.00	34.52	0
	ACP	LU	DMN 90 UM/ML	0877	0182	0164	20.75	18.70	0
000109944	ACT	LI1	0005-0 PCT.	1026	0165	0169	16.08	16.47	0
000109944	ACT	LI2	0025-1 PCT.	1053	0193	0197	18.33	18.71	0
000109944	ACT	LI3	0125-2 PCT.	1010	0248	0180	24.55	17.82	0
000109944	ACT	LU1	0005-0 PCT.	1128	0129	0105	11.44	9.31	0
000109944	ACT	LU2	0025-1 PCT.	0853	0177	0166	20.75	19.46	0
000109944	ACT	LU3	0125-2 PCT.	0910	0150	0161	16.48	17.69	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 613802 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	2302	0356	15.46	0
	A-C		SOLVENT	2223	0338	15.20	0
	ALI		TISSUE	2290	0330	14.41	0
	ALU		TISSUE	2164	0348	16.08	0
	ACP	LI	DMN 90 UM/ML	2592	2115	81.60	0
	ACP	LU	DMN 90 UM/ML	2499	0384	15.37	0
000109944	ACT	LI1	0005-0 PCT.	2396	0316	13.19	0
000109944	ACT	LI2	0025-1 PCT.	2288	0325	14.20	0
000109944	ACT	LI3	0125-2 PCT.	2050	0692	33.76	0
000109944	ACT	LU1	0005-0 PCT.	2094	0319	15.23	0
000109944	ACT	LU2	0025-1 PCT.	2004	0285	14.22	0
000109944	ACT	LU3	0125-2 PCT.	2070	0318	15.36	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 614205 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	2665	0131	4.92	0
	ACP	LI	DMN 90 UM/ML	2592	2115	81.60	0
000109944	ACT	LI3	0125-2 PCT.	0739	0129	17.46	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 613402 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1877	0102	5.43	0
	A-C		SOLVENT	1896	0083	4.38	0
	ALI		TISSUE	0535	0020	3.74	0
	ALU		TISSUE	1372	0066	4.81	0
	ACP	LI	AMQ 333 UG/ML	0267	0675	252.81	0
	ACP	LU	AMQ 333 UG/ML	1450	0116	8.00	0
000109944	ACT	LI1	0005-0 PCT.	0470	0035	7.45	0
000109944	ACT	LI2	0025-1 PCT.	0703	0035	4.98	0
000109944	ACT	LI3	0125-2 PCT.	0730	0026	3.56	0
000109944	ACT	LU1	0005-0 PCT.	0749	0047	6.28	0
000109944	ACT	LU2	0025-1 PCT.	1087	0060	5.52	0
000109944	ACT	LU3	0125-2 PCT.	1132	0051	4.51	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 613801 DETECTOR TA1538 SPECIES RHESUS/MONKEY DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0965	0134	13.89	0
	A-C		SOLVENT	1011	0134	13.25	0
	ALI		TISSUE	0838	0121	14.44	0
	ALU		TISSUE	1065	0107	10.05	0
	ACP	LI	ANTH 67 UG/ML	0714	2141	299.86	0
	ACP	LU	ANTH 67 UG/ML	0966	0129	13.35	0
000109944	ACT	LI1	0005-0 PCT.	0506	0079	15.61	0
000109944	ACT	LI2	0025-1 PCT.	0713	0109	15.29	0
000109944	ACT	LI3	0125-2 PCT.	0739	0105	14.21	0
000109944	ACT	LU1	0005-0 PCT.	0518	0076	14.67	0
000109944	ACT	LU2	0025-1 PCT.	0787	0129	16.39	0
000109944	ACT	LU3	0125-2 PCT.	0903	0127	14.06	0

Litton BIONETICS

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 614202 DETECTOR 0000D4 SPECIES RHESUS/MONKEY DATE - 05/26/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1080	0191	0182	17.69	16.85	0
	A-C		SALINE	1095	0191	0176	17.44	16.07	0
	ALI		TISSUE	0870	0195	0157	22.41	18.05	0
	ALU		TISSUE	0999	0196	0166	19.62	16.62	0
	ACP	LI	DMN 90 UM/ML	1035	0694	0209	67.05	20.19	0
	ACP	LU	DMN 90 UM/ML	1064	0180	0189	16.92	17.76	0
000109944	ACT	LI1	0005-0 PCT.	0783	0135	0117	17.24	14.94	4
000109944	ACT	LI2	0025-1 PCT.	1062	0179	0130	16.85	12.24	4
000109944	ACT	LI3	0125-2 PCT.	0748	0178	0130	23.80	17.38	0
000109944	ACT	LU1	0005-0 PCT.	0910	0173	0163	19.01	17.91	0
000109944	ACT	LU2	0025-1 PCT.	0902	0162	0131	17.96	14.52	0
000109944	ACT	LU3	0125-2 PCT.	0862	0168	0132	19.49	15.31	0